Macrophage membrane potential changes associated with $\gamma 2b/\gamma 1$ Fc receptor–ligand binding

(receptor cross-linking/immunocomplexes/monoclonal antibodies/tetraphenylphosphonium/depolarization)

JOHN DING-E YOUNG*, JAY C. UNKELESS*, H. RONALD KABACK†, AND ZANVIL A. COHN*

*The Rockefeller University, New York, New York 10021; and †Roche Institute of Molecular Biology, Nutley, New Jersey 07110

Contributed by Zanvil A. Cohn, November 29, 1982

ABSTRACT We have studied the effects of specific ligands of the receptor for the IgG Fc fragment (FcR) on the membrane potential ($\Delta\Psi$) of the macrophage cell line J774 by the [^3H]tetraphenylphosphonium ion equilibration technique. We observe a membrane depolarization with binding of FcR ligands that is dependent on the degree of receptor crosslinking. Binding of the FcR by monovalent ligands is not sufficient to induce a significant drop in $\Delta\Psi$, but a sustained depolarization lasting ≈ 20 min occurs with insoluble multivalent ligands. This FcR-mediated depolarization can be inhibited by substitution of Na^+ from the cell incubation medium with monovalent choline cation, indicating that depolarization is due to Na^+ influx into the cell. The extracellular Ca^{2+} does not play a significant role in membrane depolarization. The depolarization response is not triggered by monoclonal antibodies directed against three other major macrophage surface antigens. The cell depolarization mediated by FcR ligands is followed by a prolonged hyperpolarization that can be partially blocked by ouabain and quinine, indicating that the hyperpolarization response is a result of a combination of a Na^+ , K^+ -ATPase activity and a Ca^{2+} -activated K^+ conductance. These data support our hypothesis that the mouse macrophage IgG FcR is a ligand-dependent ion channel.

The binding of immunocomplexes to the macrophage receptor for the Fc fragment (FcR) results in the internalization of the complexes (1, 2) and the secretion of arachidonic acid metabolites (3, 4), activated oxygen intermediates (5, 6), and hydrolytic enzymes (5, 7). Like many other receptor–ligand interactions, the initial signal transmitted to the cell by the receptor and the subsequent steps leading to the activation of the host defense mechanisms are unclear.

Our interest in FcR-mediated events stemmed from the association of early membrane potential ($\Delta\Psi$) changes with the activation of leukocytes by concanavalin A, phorbol myristate acetate, and zymosan (8–12). We hypothesized that the interaction of immunocomplexes with FcRs might result in the opening of an ion channel. Because we have both monoclonal antibodies and the purified murine FcR with IgG2b and IgG1 specificities ($\gamma 2b/\gamma 1$ FcR) (2, 13, 14), a rigorous test of this hypothesis was possible.

We now report $\Delta\Psi$ changes, measured by the accumulation of [^3H]tetraphenylphosphonium ion ([^3H]Ph $_4\text{P}^+$), in the murine macrophage cell line J774 incubated with a variety of ligands. Immunocomplexes and the monoclonal antibody 2.4G2 directed against $\gamma 2b/\gamma 1$ FcR both mediate a striking cellular depolarization, the extent and duration of which is related to the degree of crosslinking of receptors. This information together with our demonstration that ion flow is a property of purified FcR in lipid vesicles (15) and planar bilayers (unpublished data)

supports our contention that the FcR is a ligand-dependent ion channel.

MATERIALS AND METHODS

Cells. The J774 macrophage cell line was grown in spinner cultures with Dulbecco's modified Eagle's medium supplemented with 5% heat-inactivated fetal bovine serum (13). Cells used were in logarithmic phase of growth between $1\text{--}3 \times 10^5$ cells per ml.

Reagents. Low- K^+ buffer consisted of 20 mM Hepes, 8.5 mM Na_2CO_3 , 118 mM NaCl, 5 mM KCl, 1.8 mM CaCl_2 , 0.8 mM MgSO_4 , and 5.5 mM glucose (pH 7.4). High- K^+ buffer was prepared by substituting KCl for NaCl. Other buffers were as described in the text.

The monoclonal antibody 2.4G2 was prepared as described (13). Other monoclonal antibodies (1.21J, 2D2C, and 2E2A) directed against major membrane proteins of mouse macrophages (16) were similarly purified. 2.4G2 Fab fragments were obtained by papain digestion (13). Dinitrophenol-modified bovine serum albumin (17) (11 mol of DNP per mol of albumin; DNP $_{11}$ -albumin) was separated from free DNP by elution through a DEAE-52 column. Rabbit antibody against DNP (anti-DNP IgG) was prepared by affinity chromatography (17). Soluble immunocomplexes were formed by mixing rabbit anti-DNP IgG and DNP $_{11}$ -albumin at an IgG/DNP $_{11}$ -albumin molar ratio of 6:1. Insoluble immunocomplexes were formed by mixing equal volumes of rabbit anti-horseradish peroxidase (HRP) antiserum (courtesy of R. Steinman, The Rockefeller University), with 0.25 mg of HRP per ml of phosphate-buffered saline. After 30 min at 4°C , the complexes were collected by centrifugation (15 min; Eppendorf Microfuge) and resuspended in buffer at a HRP concentration of 1 mg/ml.

The 2.4G2 IgG Fab fragment, intact IgG, and DNP $_{11}$ -albumin were coupled to CNBr-activated Sephadex G-25, superfine 10–40 μm (14, 16). The final protein concentrations were 1.7, 2.0, and 3.2 mg of protein per ml of packed gel, respectively. Immunocomplexes were formed on DNP $_{11}$ -albumin-modified Sephadex by incubation (30 min at 4°C) of 40 μg of anti-DNP IgG with 100 μl of packed Sephadex in a final volume of 200 μl .

Cell Sizing. The cell volume obtained by using [^3H]H $_2\text{O}$ and [^{14}C]sorbitol (18) to measure total and extracellular spaces, respectively, was comparable ($\pm 10\%$) to the cell volume obtained by means of a Coulter Counter (Coulter Channelyzer, Model H $_4$; Coulter). The latter allowed rapid measurement of cell volume before each experiment, which was necessary because the cell volume was sensitive to the time in suspension, density, type

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: $\Delta\Psi$, membrane potential; DNP, dinitrophenyl; FcR, Fc fragment receptor; $\gamma 2b/\gamma 1$ FcR, IgG $\gamma 2b/\gamma 1$ FcR; HRP, horseradish peroxidase; [^3H]Ph $_4\text{P}^+$, [^3H]tetraphenylphosphonium ion; DNP $_{11}$ -albumin, DNP-modified bovine serum albumin (molar ratio, 11:1); DNP $_{11}$ -albumin-Sephadex, Sephadex G-25 beads covalently modified with DNP $_{11}$ -albumin; anti-DNP IgG, rabbit antibody against DNP.

of buffer, pH of the medium, and temperature. The cell volumes in low- and high- K^+ buffers were 1.50 ± 0.3 and $1.36 \pm 0.1 \mu\text{l}$ per 10^6 cells, respectively.

[^3H]Ph $_4\text{P}^+$ Uptake and Membrane Potential. Cells were washed twice in low- K^+ buffer at 4°C , resuspended at 4×10^7 cells per ml and agitated at 37°C . To initiate the reaction, 1 vol of cell suspension was diluted into 9 vol of low- or high- K^+ buffer containing [^3H]Ph $_4\text{P}^+$ as noted. After equilibration of the cells for 20 min, reagents were added to the reaction mixture in small aliquots not exceeding 1/20 of the total reaction volume. At intervals, 0.1-ml aliquots were filtered rapidly through glass microfiber filters (934-AH, Whatman), and the filters then were washed with 4 ml of low- or high- K^+ buffer. Radioactivity retained on the filters was assayed by liquid scintillation spectrometry in Hydrofluor (National Diagnostics, Somerville, NJ).

The [^3H]Ph $_4\text{P}^+$ that partitioned into the cells in low- K^+ buffer was corrected for nonspecific accumulation by subtraction of [^3H]Ph $_4\text{P}^+$ taken up in high- K^+ buffer. These corrected values were converted into concentrations of [^3H]Ph $_4\text{P}^+$ by using the intracellular volumes, and the results were then converted into millivolts of membrane potential by using the Nernst equation:

$$\Delta\psi = \frac{2.3 RT}{F} \log \frac{[\text{Ph}_4\text{P}^+]_{\text{in}}}{[\text{Ph}_4\text{P}^+]_{\text{out}}}, \quad [1]$$

with $[\text{Ph}_4\text{P}^+]_{\text{in}} = [\text{Ph}_4\text{P}^+]_{\text{in, low-}K^+} - [\text{Ph}_4\text{P}^+]_{\text{in, high-}K^+}$, as described (18); $2.3 RT/F$ is -61 mV at 37°C . Trypan blue exclusion was used for viability control, and further correction of $\Delta\psi$ was made for the number of dead cells in our preparation. In all experiments, viability always exceeded 95%.

RESULTS

[^3H]Ph $_4\text{P}^+$ Uptake by J774 Cells. We used the macrophage cell line J774 as a source of large numbers of a homogeneous

FIG. 1. Calibration curves for [^3H]Ph $_4\text{P}^+$. (a) J774 cells (4×10^6 cells per ml) were incubated with $20 \mu\text{M}$ [^3H]Ph $_4\text{P}^+$ (0.125 Ci/mmol ; $1 \text{ Ci} = 3.7 \times 10^{10} \text{ Bq}$) in low- K^+ (●) and high- K^+ (○) buffers; the time course was initiated with the addition of cells. (b) Uptake of [^3H]Ph $_4\text{P}^+$ by cells incubated with different concentrations of [^3H]Ph $_4\text{P}^+$ in low- K^+ (●) and high- K^+ (○) buffers. Time points represent values of [^3H]Ph $_4\text{P}^+$ accumulated after a 25-min incubation.

phagocytic cell population. The cell-associated [^3H]Ph $_4\text{P}^+$ reached equilibrium after 10 min in both low- and high- K^+ buffers (Fig. 1a). The amount of cell-associated [^3H]Ph $_4\text{P}^+$ was proportional to cell number between $1\text{--}5 \times 10^6$ cells per ml (data not shown)

FIG. 2. Effect of $\gamma 2b/\gamma 1$ FcR ligands on membrane potential. Cells (4×10^6 cells per ml) were incubated with $20 \mu\text{M}$ [^3H]Ph $_4\text{P}^+$ for 25 min before addition of ligands (○). Control cells received no additional treatment (●). (a) 2.4G2 IgG at $0.1 \mu\text{M}$ ($n = 6$). (b) 2.4G2 Fab fragment at $5 \mu\text{M}$ ($n = 3$). (c) DNP $_{11}$ -albumin complexed with rabbit anti-DNP IgG (1:6 molar ratio) with IgG at $1 \mu\text{M}$ ($n = 3$). (d) DNP $_{11}$ -albumin-Sephadex ($20 \mu\text{l}$) complexed with anti-DNP IgG ($n = 3$). (e) 2.4G2 Fab-Sephadex (1.7 mg of 2.4G2 Fab per ml of Sephadex; $40 \mu\text{l}$ of particles per ml) ($n = 3$). (f) HRP complexed with rabbit anti-HRP ($40 \mu\text{g}$ of HRP) ($n = 2$).

Table 1. Dose dependence of $\Delta\Psi$ changes induced by 2.4G2 IgG

2.4G2, nM	Depolarization		Maximal hyperpolar- ization, mV
	Maximum, mV	Duration, min	
5	3.3 $\pm$ 0.2	2.5	12.7 $\pm$ 0.5
10	4.2 $\pm$ 0.4	3.5	12.7 $\pm$ 0.7
100	6.1 $\pm$ 0.6	4.5	13.2 $\pm$ 0.7
1,000	7.1 $\pm$ 0.9	7.0	15.9 $\pm$ 1.2
2,000	6.9 $\pm$ 0.4	7.2	16.4 $\pm$ 0.5
5,000	7.1 $\pm$ 0.5	7.0	16.1 $\pm$ 1.1

J774 cells (4×10^6 cells per ml) were incubated with 20 μ M [3 H]Ph $_4$ P $^+$ for 20 min before addition of 2.4G2 IgG at the various concentrations indicated. Data represent means $\pm$ SEM of six experiments.

and increased linearly with the [3 H]Ph $_4$ P $^+$ concentration from 2 to 50 μ M (Fig. 1b). The J774 cell resting $\Delta\Psi$, calculated with the Nernst equation and cell volumes determined as described in *Materials and Methods*, was -14.1 ± 0.3 mV, a value comparable to that reported by Gallin *et al.* for guinea pig peritoneal macrophages (19). In most subsequent experiments, cell density in the reaction mixture was 4×10^6 cells per ml, and concentration of [3 H]Ph $_4$ P $^+$ was 20 μ M.

Effect of Ligands for the FcR on $\Delta\Psi$. After the addition of 0.1 μ M 2.4G2 IgG, cells showed a biphasic change in $\Delta\Psi$ (Fig. 2a). Within 20 sec of addition of antibody, the cells depolarized to -7 mV, followed after ≈ 7 min by a prolonged hyperpolarization of the cell $\Delta\Psi$ to a value of -30 mV. Both the maximal depolarization values and duration of this rapid decrease in $\Delta\Psi$ were dependent upon the dose of 2.4G2 IgG (Table 1). Saturation of the response occurred at a concentration of 2.4G2 IgG above 1 μ M. The monovalent 2.4G2 Fab fragment, at concentrations up to 5 μ M did not depolarize the cells but still induced a prolonged hyperpolarization, which occurred after ≈ 3 min and attained a maximum of -24 mV (Fig. 2b). Soluble immunocomplexes were tested in the same system. Initial binding studies showed optimal binding to macrophages at a DNP $_{11}$ -albumin/anti-DNP IgG molar ratio of 1:6. Preincubation of these soluble immunocomplexes at an IgG concentration of 1 μ M resulted in changes in $\Delta\Psi$ qualitatively similar to those produced by 2.4G2 IgG (Fig. 2c).

The effect of an insoluble, multivalent FcR ligand on $\Delta\Psi$ was tested by preincubating J774 cells with Sephadex beads coated with immune complexes. Incubation with DNP $_{11}$ -albumin-Sephadex alone was done in parallel as a control. The immunocomplexes bound to Sephadex produced a prolonged depolarization to -3 mV, which lasted ≈ 20 min, after which repolarization occurred with only a small overshoot of the resting $\Delta\Psi$ (Fig. 2d). Similar results were obtained by using insoluble immunocomplexes formed by incubation of rabbit anti-HRP antiserum with HRP (Fig. 2f).

Table 2. Na $^+$ dependence of $\Delta\Psi$ changes induced by 2.4G2 IgG

Cho $^+$ /Na $^+$, mM	$\Delta\Psi$, mV		
	Resting value	Maximal depolar- ization	Maximal hyperpolar- ization
0/135	14.2 $\pm$ 0.3	7.2 $\pm$ 0.3	15.5 $\pm$ 0.7
70/65	14.1 $\pm$ 0.4	7.1 $\pm$ 0.5	13.3 $\pm$ 0.5
30/105	13.9 $\pm$ 0.5	4.3 $\pm$ 0.2	7.5 $\pm$ 0.6
135/0	14.0 $\pm$ 0.2	2.0 $\pm$ 0.3	3.8 $\pm$ 0.4

J774 cells (4×10^6 cells per ml) were incubated in different buffers as indicated for 10 min, followed by 20 μ M [3 H]Ph $_4$ P $^+$ for 20 min before addition of 0.5 μ M 2.4G2 IgG. Data represent means $\pm$ SEM of six experiments. Cho $^+$, choline cation.

Table 3. Effect of Ca $^{2+}$ depletion on $\Delta\Psi$ changes induced by 2.4G2 IgG

CaCl $_2$, mM	$\Delta\Psi$, mV		
	Resting value	Maximal depolar- ization	Maximal hyperpolar- ization
3	14.2 $\pm$ 0.3	7.0 $\pm$ 0.4	15.2 $\pm$ 1.1
0 $\pm$ 1 mM EGTA	13.5 $\pm$ 0.4	6.7 $\pm$ 0.6	13.3 $\pm$ 0.6

J774 cells (4×10^6 cells per ml) were treated with media containing or free of Ca $^{2+}$ for 10 min before equilibration with [3 H]Ph $_4$ P $^+$ for 20 min, after which 0.5 μ M 2.4G2 IgG was added. Data represent means $\pm$ SEM of seven experiments.

Whereas the monomeric 2.4G2 Fab fragment resulted in minimal depolarization (Fig. 2b), incubation of J774 cells with the 2.4G2 Fab-modified Sephadex resulted in the same dramatic depolarization observed with either Sephadex coated with immunocomplexes or insoluble antigen-antibody precipitate (Fig. 2e). Intact 2.4G2 IgG bound to Sephadex gave a similar result (data not shown). These results indicate that the extent and duration of depolarization are dependent on multivalent cross-linking of the FcR.

Role of Cations in Depolarization. The depolarization induced by 2.4G2 IgG or immunocomplexes may be due to influx of Na $^+$. To demonstrate this, we performed ion substitution experiments. Substitution of Na $^+$ by monovalent choline cation did not affect the resting $\Delta\Psi$ of the cells but decreased the extent of depolarization by 80% (Table 2). The depolarization in response to immunocomplexes of cells maintained in choline cation-containing medium for 20 min was restored promptly after resuspension of the cells in Na $^+$ -containing medium. Cells already depolarized by suspension in high-K $^+$ buffer did not respond with further change in $\Delta\Psi$ after addition of different ligands. The cells could recover their normal $\Delta\Psi$ if exposure to high-K $^+$ buffer did not exceed 20 min (data not shown). The depolarization in response to 2.4G2 IgG was not Ca $^{2+}$ dependent, nor was the resting $\Delta\Psi$ significantly altered by incubation for 30 min in Ca $^{2+}$ -free medium containing 1 mM EGTA (Table 3).

Mechanisms of Hyperpolarization. A Na $^+$ and possibly Ca $^{2+}$ influx into the cells may play a role in hyperpolarization. Incubation of cells in choline for 20 min decreased the hyperpolarization induced by 2.4G2 IgG by 80%. Exposure to Ca $^{2+}$ -free medium for 30 min decreased the 2.4G2 IgG-induced hyperpolarization by 10% (Table 3). To investigate further the roles of Na $^+$ and Ca $^{2+}$ in hyperpolarization, the effects of ouabain, which blocks Na $^+$, K $^+$ -ATPase (20, 21), and quinine, which blocks Ca $^{2+}$ -activated K $^+$ conductance (22), were tested. The resting $\Delta\Psi$ of macrophages was not affected by incubation with 1 mM

Table 4. Effects of ouabain and quinine on $\Delta\Psi$ changes induced by 2.4G2 IgG

Treatment	$\Delta\Psi$, mV		
	Resting value	Maximal depolar- ization	Maximal hyperpolar- ization
Control	14.2 $\pm$ 0.4	6.9 $\pm$ 0.3	15.3 $\pm$ 0.6
Ouabain, 1 mM	14.0 $\pm$ 0.4	6.6 $\pm$ 0.5	4.5 $\pm$ 0.3
Quinine, 0.1 mM	13.7 $\pm$ 0.2	6.5 $\pm$ 0.6	12.2 $\pm$ 0.4
Ouabain, 1 mM + quinine, 0.1 mM	13.5 $\pm$ 0.6	6.6 $\pm$ 0.4	2.4 $\pm$ 0.2

J774 cells (4×10^6 cells per ml) were treated as indicated for 10 min followed by equilibration with 20 μ M [3 H]Ph $_4$ P $^+$ for 20 min before addition of 2.4G2 IgG (0.5 μ M). Data represent means $\pm$ SEM of four experiments.

ouabain or 0.1 mM quinine for a period of 30 min (Table 4). The depolarization response induced by 0.1 μ M 2.4G2 IgG was not affected by either or both drugs at the concentrations used for the experiments. However, the hyperpolarization was inhibited by 70% in the presence of ouabain, 20% with quinine, and 85% in the presence of both drugs.

Effects of Other Monoclonal Antibodies on $\Delta\Psi$. We tested the effects of three other monoclonal antibodies (1.21J, 2D2C, and 2E2A) that recognize major membrane proteins of mouse macrophages (16). At 5 μ M, all three initiate hyperpolarization within 1 min with no initial depolarization (data not shown). 1.21J increased the $\Delta\Psi$ to -28 mV, whereas 2D2C and 2E2A, at the same concentrations, hyperpolarized the cells to -23 mV and with shorter duration than that induced by 1.21J. The effects produced by these monoclonal antibodies were completely inhibited by treating the cells with 1 mM ouabain for 30 min prior to addition of the antibodies.

DISCUSSION

The binding of immunocomplex-coated particles to FcRs of macrophages and neutrophils triggers a variety of physiological events, including phagocytosis and secretion of cidal agents and mediators of vascular permeability. We have used the lipophilic cation [3 H]Ph $_4$ P $^+$ to measure changes in $\Delta\Psi$ after binding of immunocomplexes and monoclonal antibodies to J774 macrophages. The initial signal delivered by the ligand-occupied γ 2b/ γ 1 FcR is a rapid membrane depolarization, observed as early as 20 sec after binding of the ligand. With the use of the thiodicarbocyanine dye Di-S-C $_3$ (5), membrane depolarization responses are observed immediately after the addition of immunocomplexes (unpublished observations). Immunocomplex-mediated depolarization is reduced when Na $^+$ is replaced by monovalent choline cation and probably represents a Na $^+$ influx into the cell. We believe that this initial signal activates subsequent cellular responses, which are determined in large part by the state of "activation" of the macrophages (7).

The response of macrophages to immunocomplexes is "graded" depending on the extent of crosslinking of the receptors. Thus, although soluble immunocomplexes will be rapidly cleared by adsorptive endocytosis, they will not suffice to trigger the secretion of arachidonate metabolites (unpublished data). Similarly, the extent and duration of the depolarization response of J774 cells is strikingly dependent upon ligand crosslinking. Insoluble immunocomplexes or 2.4G2 Fab-Sephadex result in complete depolarization lasting for about 20 min, soluble complexes or 2.4G2 IgG result in a transient depolarization, and 2.4G2 Fab has no depolarizing effect on the cells.

The depolarization we have observed with FcR ligands appears to be specific for the FcR. We have examined the $\Delta\Psi$ changes mediated by binding to J774 cells of three other monoclonal antibodies that recognize major surface antigens, which are present in comparable amounts to γ 2b/ γ 1 FcR. These antibodies trigger a hyperpolarization of the cells, which also is observed after a variable lag period with cells incubated with 2.4G2 Fab fragment, 2.4G2 IgG, and soluble immunocomplexes. The hyperpolarization is due largely to activation, by an unknown mechanism, of the Na $^+$, K $^+$ -ATPase electrogenic pump because from 70 to 100% of the hyperpolarization can be blocked by ouabain. Ouabain also blocks the hyperpolarization that results from Na $^+$ influx induced by the Na $^+$ -H $^+$ exchanger monensin (21). A lesser inhibition (20%) of the hyperpolarization response is mediated by quinine, which blocks a Ca $^{2+}$ -mediated K $^+$ conductance (22).

The concentration of free intracellular Ca $^{2+}$ plays a central role in stimulation of secretion (23) and the regulation of cyto-

skeletal proteins involved in phagocytosis (24, 25). In the present case, Ca $^{2+}$ permeability does not appear to play a major role in $\Delta\Psi$ changes mediated by FcR-ligand interaction, and macrophages and monocytes are capable of ingestion of antibody-coated particles in Ca $^{2+}$ -free medium (26, 27). An influx of Na $^+$ into the cell, triggered by the FcR, may result in calcium release from intracellular stores (28) and, indeed, cytochemical observations implying displacement of membrane-bound Ca $^{2+}$ from the cytoplasmic side of neutrophil membranes after perturbation by particulate stimuli (opsonized zymozan, immunocomplex precipitates, and concanavalin A-Sepharose) have been reported (29).

The results presented here show interesting analogies with the basophil/mast cell IgE FcR (30), which has mobility on polyacrylamide gel electrophoresis similar to that of the mouse γ 2b/ γ 1 FcR (14) and the human neutrophil FcR (31). Bridging of mouse γ 2b/ γ 1 FcR is necessary for optimal $\Delta\Psi$ response to FcR ligands. Bridging of IgE FcR is also required for secretion of histamine from basophils and mast cells (32–34). In addition, the magnitude of this secretory response from rat basophilic leukemia cells is also dependent on the extent of crosslinking of the IgE FcR (35). However, the Ca $^{2+}$ influx into basophils/mast cells, which is associated with occupied IgE FcR (36), does not appear to be a major factor for ligand-dependent depolarization mediated by γ 2b/ γ 1 FcR.

FcR ligand-induced cell depolarization is compatible with our hypothesis that the mouse macrophage FcR is a ligand-dependent ion channel. However, the exact mechanism underlying cell depolarization mediated by FcR ligands cannot be resolved through equilibration of cells with voltage-sensitive probes. In subsequent papers, we show that the ion flow associated with binding of the γ 2b/ γ 1 FcR is a property of the purified receptor itself.

We acknowledge Dr. M. L. Garcia for her assistance with the [3 H]-Ph $_4$ P $^+$ uptake experiments and Dr. S. Silverstein for helpful discussions. This work was supported in part by Grant AI-14603 from the National Institutes of Health. J.C.U. is the recipient of Faculty Research Award FRA 205 from the American Cancer Society. J.D.-E.Y. is a fellow of the National Research Council, Brazil, and the recipient of a predoctoral fellowship from the National Institutes of Health.

1. Silverstein, S. C., Steinman, R. M. & Cohn, Z. A. (1977) *Annu. Rev. Biochem.* **46**, 669–722.
2. Unkeless, J. C., Fleit, H. & Mellman, I. S. (1981) *Adv. Immunol.* **31**, 247–270.
3. Rouzer, C. A., Scott, W. A., Kempe, J. & Cohn, Z. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4279–4282.
4. Scott, W. A., Zrike, J. M., Hamill, A. L., Kempe, J. & Cohn, Z. A. (1980) *J. Exp. Med.* **152**, 324–335.
5. Nathan, C. F., Murray, H. W. & Cohn, Z. A. (1980) *N. Engl. J. Med.* **303**, 622–626.
6. Nathan, C. F. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 2206–2211.
7. Cohn, Z. A. (1978) *J. Immunol.* **121**, 813–816.
8. Korchak, H. M. & Weissmann, G. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3818–3822.
9. Korchak, H. M. & Weissmann, G. (1980) *Biochim. Biophys. Acta* **601**, 180–194.
10. Miles, P. R., Bowman, L. & Castranova, V. (1981) *J. Cell. Physiol.* **106**, 109–117.
11. Jones, G. S., VanDyke, K. & Castranova, V. (1981) *J. Cell. Physiol.* **106**, 75–83.
12. Whitin, J. C., Chapman, C. E., Simons, E. R., Chovaniec, M. E. & Cohen, H. J. (1980) *J. Biol. Chem.* **255**, 1874–1878.
13. Unkeless, J. C. (1979) *J. Exp. Med.* **150**, 580–596.
14. Mellman, I. S. & Unkeless, J. C. (1980) *J. Exp. Med.* **152**, 1048–1069.
15. Young, J. D., Unkeless, J. C., Kaback, H. R. & Cohn, Z. A. (1983) *Proc. Natl. Acad. Sci. USA* **80**, in press.
16. Mellman, I. S., Steinman, R. M., Unkeless, J. C. & Cohn, Z. A. (1980) *J. Cell Biol.* **86**, 712–722.

17. Unkeless, J. C. (1977) *J. Exp. Med.* **145**, 931–947.
18. Lichtshtein, D., Kaback, H. R. & Blume, A. J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 650–654.
19. Gallin, E. K., Wiederhold, M. L., Lipsky, P. E. & Rosenthal, A. S. (1975) *J. Cell. Physiol.* **86**, 653–662.
20. Glynn, I. M. & Karlish, S. J. O. (1975) *Annu. Rev. Physiol.* **37**, 13–55.
21. Lichtshtein, D., Dunlop, K., Kaback, H. R. & Blume, A. J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2580–2584.
22. Lew, V. L. & Ferreira, H. G. (1978) in *Curr. Top. Membr. Transp.* **10**, 218–277.
23. Rubin, R. P. (1982) *Calcium and Cellular Secretion* (Plenum, New York), pp. 1–276.
24. Yin, H. L. & Stossel, T. P. (1979) *Nature (London)* **281**, 583–586.
25. Yin, H. L. & Stossel, T. P. (1980) *J. Biol. Chem.* **255**, 9490–9493.
26. Stossel, T. P. (1973) *J. Cell Biol.* **58**, 346–356.
27. Wright, S. D. & Silverstein, S. C. (1982) *J. Exp. Med.* **156**, 1149–1164.
28. Lowe, D. A., Richardson, B. P., Taylor, P. & Donatsch, P. (1976) *Nature (London)* **260**, 337–338.
29. Hoffstein, S. T. (1979) *J. Immunol.* **123**, 1395–1402.
30. Metzger, H., Goetze, A., Kanellopoulos, J., Holowka, D. & Fewtrell, C. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 8–11.
31. Fleit, H. B., Wright, S. D. & Unkeless, J. C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3275–3279.
32. Ishizaka, I. & Ishizaka, K. (1979) *J. Immunol.* **120**, 800–805.
33. Segal, D. M., Taurog, J. D. & Metzger, H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2993–2997.
34. Ishizaka, T., Hirata, F., Ishizaka, K. & Axelrod, J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1903–1906.
35. Fewtrell, C. & Metzger, H. (1980) *J. Immunol.* **125**, 701–710.